

MICROSCOPY and CELL STRUCTURE

Readings: Review pp. 69-71, and Figure 4.1 on p. 65 in your text (POHS, 5th ed.).

Introduction:

Biologists rely on many different types of microscopic techniques to find out where molecules are located in cells, and to zero in on what is happening in individual cells and in very small organisms. This laboratory exercise will introduce you to the use of the compound microscope. You will be looking at cells, cellular organelles, and small organisms, and you will learn to use micrometers to measure the sizes of microscopic structures. Biologists work with organisms that range in size over many orders of magnitude. By observing and measuring different types of cells and cellular structures, you will begin to get a practical feeling for the relative sizes of things.

LAB GOALS

Learn to use a compound microscope. Learn to use ocular and stage micrometers to accurately measure microscopic specimens. Use light microscopy to observe live specimens and cellular organelles.

Before you begin this exercise, read and work through the following sections!

Things to remember about microscopes:

- **Microscopes have loose parts.** This varies with different styles of microscopes. You need to be very careful: 1) when moving the microscope (use both hands), and 2) when removing the cover from the microscope (for example, with some microscopes, the eyepieces can catch on the microscope cover and fall off while the cover is being removed).
- **Never clean a microscope lens with anything but lens paper.** The lenses or lens coatings can be easily scratched and damaged.
- **Each specimen is mounted on a glass microscope slide for viewing.** Usually a small, thin coverslip is placed over the specimen. With wet specimens, a coverslip helps decrease the rate of evaporation. Coverslips also protect the lenses of the longer objectives from getting immersed in the specimen and improve what you can see.

● **Always begin work on each new specimen with the lowest power objective in place.** This makes work significantly easier for you and safer for the microscope. The advantage for you is that the specimen will be easier to find and easier to bring into focus. The advantage for the microscope is that the shorter low power objective will be further from your sample when in focus. Therefore, the low power objective lens is less likely to be damaged while you are searching for the correct plane of focus. The lowest power objective will generally be the shortest one. On these microscopes, it is 4X or 10X.

Total magnification = magnification_{eyepiece} x magnification_{objective}

Getting started: Focusing a specimen and initial adjustments.

A labeled drawing of the microscope you will use is provided for your reference on the last page of this exercise. Some of the models you are using may differ slightly.

Before you start:

- (1) If the microscope is not plugged in, unwrap all of the cord from around the microscope and plug it in.
- (2) Adjust the eyepieces so they are the right distance apart for your eyes.

You are provided with a prepared slide of the letter **e**. This traditional introductory “specimen” is useful, as it illustrates the inverted images observed in the microscope. Place the slide on the microscope stage under the 4X or 10X objective. Turn on the light source and adjust the intensity. You will need to readjust the light intensity as you change objectives. Check to be sure that the substage condenser is raised as far as possible. Proper adjustment of the condenser will be explained in class. Move the **e** into the beam of light, using the **mechanical stage control knobs**.

To find the plane of focus:

- (1) Looking from the side, bring the low power objective as close as possible to the stage.
- (2) Determine which way to rotate the **coarse adjustment knob** in order to move the stage away from the objective.
- (3) Next, while looking through the eyepieces, slowly move the stage down (away from the objective) until the specimen comes into focus. Use the **fine adjustment** to refine the focus.

To focus the eyepieces, close your left eye and use the **fine adjustment** knob to focus the right eyepiece. Then close your right eye and rotate the **eyepiece focus tube** to focus the left eyepiece.

Center the specimen and examine it using the 10X and 40X objectives. The specimen may be “lost” from your field of view if you don’t center it before switching to a higher power objective. On these microscopes, all of the objectives are approximately **parfocal**. This means that once you find the correct plane of focus on low power you will have to make only minor adjustments (with the fine adjustment knob) when you change objectives.

The quality of light can also be adjusted using the **iris diaphragm control lever**. As you work with different specimens, experiment with the effect this control has on the appearance of your specimen – closing the iris diaphragm will increase contrast and opening it will increase resolution. Some transparent organisms are completely invisible unless the iris diaphragm is closed. Other specimens look much better with the iris diaphragm open.

EXPERIMENTAL PROTOCOLS

Protocol 1. Calibrating the Ocular Micrometer using a Stage Micrometer.

Now that you have practiced the basics, you are ready to calibrate your microscope for making measurements. The ocular micrometer is in one eyepiece. You can use this to measure the size of your specimens in “ocular units”, but what is one “ocular unit” equivalent to in real metric units? One problem is that the value of each “unit” on the ocular micrometer changes every time you change magnification by changing the objective. You need to determine empirically how much “one unit” on the ocular micrometer is equivalent to by using a stage micrometer. A stage micrometer looks like a microscope slide and has a tiny ruler calibrated in metric units (millimeters, tenths of millimeters, etc.) etched onto it. You will determine the length (in mm) of one “ocular unit” for each objective you will be using.

Note that several different styles of ocular and stage micrometers are used in this lab; yours may not be identical to that of the student next to you. Also, be very careful with the stage micrometers (for example, don’t leave them near the edge of your workbench), as they are outrageously expensive.

Place the micrometer on the microscope stage and bring it into focus, using the lowest power objective. Rotate the eyepiece so the scale of the ocular micrometer overlaps the scale on the stage micrometer. For each objective (that is, for each level of magnification) determine the real value of “one unit” on the ocular micrometer (in mm/ocular unit), using the following relationship:

$$\frac{X \text{ mm}}{Y \text{ ocular units}} = \frac{X / Y \text{ mm}}{\text{ocular unit}}$$

For example: $0.55 \text{ mm} / 30 \text{ ocular units} = 0.018 \text{ mm} / \text{ocular unit} =$ the conversion factor for that objective

- Calibrate each of the three principal objectives, 4X, 10X, and 40X. That is, determine how many *mm/ocular unit* for each objective. Record your calculations in your laboratory notebook.
- What is the relationship between the value of one ocular unit at 4X and the value of one ocular unit at 40X?
- Use this relationship to calculate what the value of one ocular unit would be at 100X. (You will not be able to measure this directly, because the stage micrometers are too thick to observe with the 100X objective!)

Use the following relationship to determine the size of a specimen:

$$\text{size in ocular units} \times \text{conversion factor} = \text{size in mm}$$

- Express the measurements you made in the section above in microns. Remember, one micrometer or micron (μm) = 10^{-3} millimeters.
- Next, measure the width of a standard thread or wire on a prepared slide provided to you. This measurement will be a point where you can check with your instructors to see whether your calculations are correct.

Protocol 2. Observing Cells and Cellular Organelles.

Now you are ready to observe and measure the specimens described below. For each specimen, use whichever magnification makes sense for observing and measuring individual cells or organelles. Remember to always start with the lowest power objective each time you begin looking at a new specimen.

(1) *Paramecium*: a unicellular non-photosynthetic organism (singular: *Paramecium*; plural: *Paramecia*). Place a drop of water with *Paramecia* on the microscope slide. Place a coverslip over the wet mount. You will trap fewer bubbles under the coverslip if you set one edge of the coverslip into an edge of the drop of liquid on the microscope slide, and then lower the coverslip the rest of the way down. Find a *Paramecium* on your slide. There may be other organisms in the water as well. When you find a *Paramecium*, look for the contractile vacuole that fills with water and then regularly flushes it out. You may also be able to detect the cilia moving on the outside of this organism. The *Paramecia* may be swimming fairly fast. To slow the *Paramecia*, prepare another slide. This time, place a drop of the viscous liquid methylcellulose next to the drop of water with the *Paramecia* sample, and then place a coverslip over both drops.

- How long and how wide is a typical *Paramecium* cell?

(2a) *Amoeba* is also a unicellular non-photosynthetic organism. Handle this organism similarly to *Paramecium*.

- How long and how wide is an *Amoeba*?

(2b) We may have *Volvox* and/or *Anabena* available for observation. Both of these organisms are unicellular, but colonial photosynthesizers.

- How long and how wide is an individual *Volvox* or *Anabena* cell?

(3) *Elodea*, an aquatic higher plant. These cells are especially good specimens for observing chloroplasts. Also notice the cell walls. Take a leaf and mount it in a drop of water under a coverslip, as flat as possible. Try to get thin new leaves and examine the edge of a leaf.

- What are the dimensions (length and width) of the chloroplasts?

(4) Onion. This is good material for observing nuclei. Take a very thin piece of the membrane between onion layers and mount it in a drop of water under a coverslip.

- What cell parts can you observe in your preparation?
- What is the width of a typical onion cell?
- Of an onion nucleus?

(5) Bacterial cells.

(**Option 1**) There are two demonstration stations with identical preparations of heat fixed, stained bacteria. One microscope is focused at 40X and the other is focused at 100X (using oil immersion optics).

- Determine the approximate length of a bacterial cell.

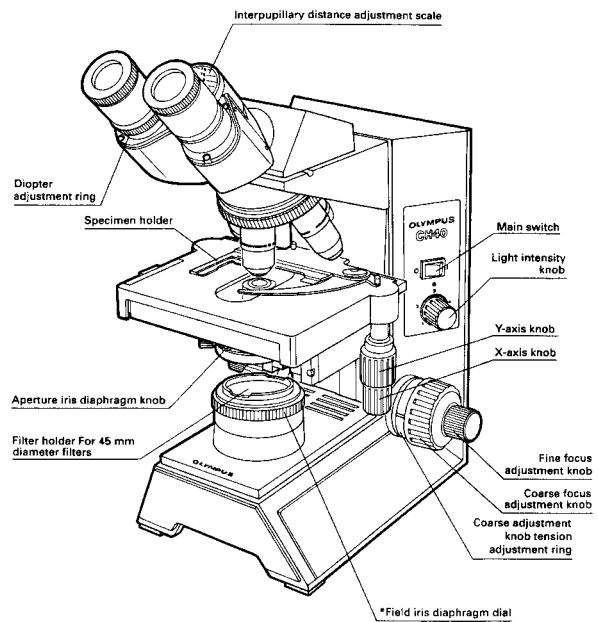
(**Option 2**) Consult Appendix B for instructions on using the oil immersion lens.

DATA ANALYSIS and WRITE UP.

There is no formal write up required for this exercise. Before you leave the laboratory, make certain you understand how to use the ocular and stage micrometers.

Compound Microscope Uses:

1. Examining whole specimens or parts of relatively small, transparent organisms such as small aquatic invertebrates, protozoa, freshwater algae, fungi, etc.
2. Examining diagnostic cross sections of plants (higher plant & moss leaves, stems), lichens, seaweeds, mushroom gills, etc.
3. General viewing of communities of microorganisms in pond scum, plankton, scrapings from rocks, gut contents, etc.



Basic Techniques:

A. Specimen preparation ("wet mounts"):

- Specimen must be in a suitable mounting medium (fresh water, salt water, methylcellulose, stain) between a slide and coverslip.
- Keep sections and mounts as thin as possible.
- Use small amounts of mixed specimens (pond scum, etc.).
- Eliminate air bubbles by lowering coverslip edge onto slide.

B. Lighting (turn on/off with switch: slider intensity control should be 6 or lower):

- EXPERIMENT with a variety of settings to make the view good for YOU!
- Substage condenser assembly should be positioned all the way up.
- Best lighting combines image resolution (clarity) and contrast (light-dark). Increase contrast by closing condenser iris (lever to right). Increase clarity by opening condenser iris (lever to left).
- Use intensity control, not condenser iris, to adjust intensity of illumination.
- After each change of objectives, readjust lighting, particularly condenser iris setting.

C. Microscope controls:

- Focusing: Start at low magnification (4X or 10X obj. lens). Start with end of objective lens just above slide. Use COARSE ADJUSTMENT to focus image. ADDITIONAL FOCUSING WITH FINE ADJUSTMENT ONLY.
- Adjust "interpupillary" distance between eyepiece tubes to obtain perfect binocular vision.
- Make sure both eyes are in focus together. Set each eyepiece tube so the line is on your interpupillary distance. If your eyes don't focus together, either wear eyeglasses or independently focus each eyepiece, as follows: Focus on a dark part of the image; close left eye and use FINE ADJUSTMENT to focus right eye; close right eye and rotate the left eyepiece adjustment to focus left eye.
- Center image before changing magnification. Rotate lens turret to next objective lens. Use fine adjustment only to focus! Readjust lighting.
- Oil must be used on the end of the 100X objective (oil immersion) lens. Otherwise, the image produced will be of lower quality than that by the 45X objective lens. Clean off lens with lens tissue when done.
- Total magnification = magnification_{eyepiece} x magnification_{objective}

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